Early Adaptation of Thyrotropin and Thyroglobulin Secretion to Experimentally Decreased Iodine Supply in Man

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Five healthy male volunteers (aged 25 to 28 years) were studied both after 4 weeks of treatment with 200 μg iodine/d orally (PO) and following experimental iodine depletion by treatment with 3 x 300 mg perchlorate/d PO over a 4-week period, in an attempt to better define the early adaptive responses to an alteration in iodine supply in thyroid function. Intrathyroidal iodine, serum triiodothyronine (T_3), free T_3 (FT_3), thyroxine (T_4), free T_4 (FT_4), reverse T_3 (rT_3), thyroxine-binding globulin (TBG), thyroglobulin (Tg), and thyrotropin (TSH) levels (10-minute sampling over 24 hours) were measured at the end of iodine administration and at the end of perchlorate treatment. Thyroid volume was determined by sonography, and iodine content was determined by fluorescence scintigraphy. TSH pulses were analyzed by computer-assisted programs. Comparing both experimental situations, perchlorate treatment significantly reduced intrathyroidal iodine concentration (4.0 ± 1.2 to 2.0 ± 1.2 nmol/mL, $P < .05$), but thyroid volume and total serum T_4, T_3, FT_4, and TBG levels were not altered. Mean 24-hour serum TSH levels (1.8 ± 0.3 to 1.0 ± 0.3 mU/L, $P < .001$), amount of TSH secreted/pulse (0.6 ± 0.1 to 0.3 ± 0.1 mU/L, $P < .001$), and FT_4 levels (15.7 ± 1.7 to 14.3 ± 1.4 pmol/L, $P < .005$) were significantly diminished, whereas Tg levels (18.6 ± 10.0 to 25.1 ± 14.0 ng/mL, $P < .01$) were significantly increased. Thyroid-specific antibodies were normal and were not altered by treatment. These data suggest a higher sensitivity of the thyroid to TSH in the early adaptation to iodine depletion; thus, less TSH is sufficient to maintain normal thyroid function.

THE INFLUENCE OF dietary iodine deficiency on thyroid growth and goiter formation has been thoroughly documented,[1] but the mechanisms involved in this pathophysiological adaptation are still the subject of considerable controversy. Thyrotropin (TSH), via cyclic adenosine monophosphate (cAMP)-dependent pathways, has been shown to directly stimulate specific thyroid functions such as iodine trapping, thyroid peroxidase, and thyroglobulin (Tg) gene expression.[2-5] In many species, including humans, TSH directly mediates thyrocyte proliferation and differentiation, but these effects have been disputed in other species.[5] Severe iodine deficiency decreases thyroid hormone formation, and it has been argued that an accompanying increase in circulating TSH serum levels stimulates goiter growth.[6-8] This pathophysiological link between thyroidal iodine supply and TSH-mediated thyroid proliferation has been the basis of the treatment of endemic goiter with thyroid hormones. However, an increased TSH secretion has never convincingly been demonstrated in patients with endemic goiter grades I and II, suggesting that the increase in TSH serum levels occurs late in the disease.[7,9,10] Bray[11] explained this discrepancy by an increased sensitivity of the thyroid to TSH in iodine deficiency. With the recent discovery that TSH is released in pulses,[12,13] this increased sensitivity may, as an alternative hypothesis, be explained by changes in the pattern of pulsatile TSH release. Iodine deficiency may stimulate thyrocyte proliferation not by an increase in circulating serum levels of TSH, but by altering the pattern of pulsatile TSH release. In the present study, we tested healthy male volunteers for potential early changes in the temporal pattern and absolute serum concentration of TSH in response to a decrease in iodine supply to the thyroid.

SUBJECTS AND METHODS

Five healthy male subjects (aged 25 to 28 years) participated in the study after giving written informed consent. Special care was taken to ensure normal thyroid function by measuring total and free thyroxine (T_4 and FT_4) and triiodothyronine (T_3 and FT_3) (Corning, Giessen, Germany), and by determination of thyroid-specific antibody levels (thyroid peroxidase antibodies, antithyroglobulin antibodies, and TSH-receptor antibodies; Henning, Berlin, Germany). Nodular thyroid changes were excluded by high-frequency sonography (7.5 MHz), and thyroid size was evaluated by ultrasound. During the first 4 weeks of the study, 200 μg/d iodine (Jodid 100, Merck, Darmstadt, Germany) was administered orally (PO), and at the end of this period blood was sampled under standardized conditions (sleep occurred between 12:00 AM and 7:00 AM, meals were at fixed times) every 10 minutes over 24 hours. On the following day, intrathyroidal iodine content was determined as previously described[14] by fluorescence scintigraphy (Fluorescence Scanner System 4202, EG&G Instruments, München, Germany; mounted on a Picker Magnascanner 500, Picker, Germany) using americium 241 as the source of radiation.

After iodine supplementation was discontinued, the subjects were treated with 3 × 300 mg perchlorate/d PO (Irenat, Tropon, Köln, Germany) to induce a state of iodine depletion. At the end of a 4-week period of iodine depletion, the initial 24-hour blood-sampling protocol and the evaluation of hormone levels, thyroid-specific antibody titers, intrathyroidal iodine content, and thyroid size were repeated.

T_3, T_4, FT_3, FT_4, and thyroxine-binding globulin (TBG) serum levels were measured as a mean of six serum samples obtained in 6-hour intervals by commercially available radioimmunoassay kits (Corning, Giessen, Germany; Behring, Marburg, Germany). Serum reverse T_3 (rT_3) concentrations were measured in the same serum samples, as previously described.[15] Tg serum levels were determined in 30-minute intervals, and in each 10-minute sample TSH was measured by our previously described method,[16] using commercially available immunometric kits (Henning, Berlin, Germany). Both rhythms of an individual were tested in the same set of

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assays. The maximal intra-assay or inter-assay coefficient of variation of all assays was 8.7%.

To determine pulsatile TSH release a recently described, computer-assisted program for pulse analysis, DESADE, was used, with a calculated rate of false-positive pulses of less than 1%.[16] Cross-correlation between TSH and Tg rhythms, using 30-minute intervals for both hormones, was performed using a computer-assisted program.[17] Statistical evaluation was performed by paired Student's *t* test; values are presented as the mean ± SD.

RESULTS

Mean thyroid volume in the volunteers was not significantly different at the end of the iodine supplementation period and following perchlorate treatment. In contrast, intrathyroidal iodine concentration was significantly decreased by iodine depletion. The circulating serum concentrations of thyroid hormones were not significantly altered by either treatment. Only serum FT_4 levels decreased slightly but significantly. No changes were seen in T_3/T_4 or FT_3/FT_4 ratios, thyroid-specific antibody titers, or TBG serum levels (Table 1).

Serum levels of Tg almost doubled following iodine depletion, and this effect was visible during the entire 24-hour period (Fig 1, Table 1). Data analysis by both computer-assisted programs showed no pulsatile release pattern of Tg, and no clear circadian changes of Tg were found. The pattern of TSH secretion during iodine supplementation showed the expected pulsatile and circadian variations (Table 2). Following perchlorate treatment, mean serum levels of TSH were significantly diminished, but the circadian pattern was still present (Fig 1). The analysis of the pulsatile release pattern demonstrated a significant reduction in pulse amplitude in each rhythm, but the number and distribution of TSH pulses remained unchanged (Table 2); no significant cross-correlation between Tg and TSH rhythms could be detected.

DISCUSSION

The importance of iodine deficiency for goiter formation is undisputed. TSH may mediate these effects by its interaction with iodine uptake and organification and its role in thyroid growth regulation. However, the pathophysiological changes in TSH occurring during the transition from normal dietary iodine supply to the supply expected in

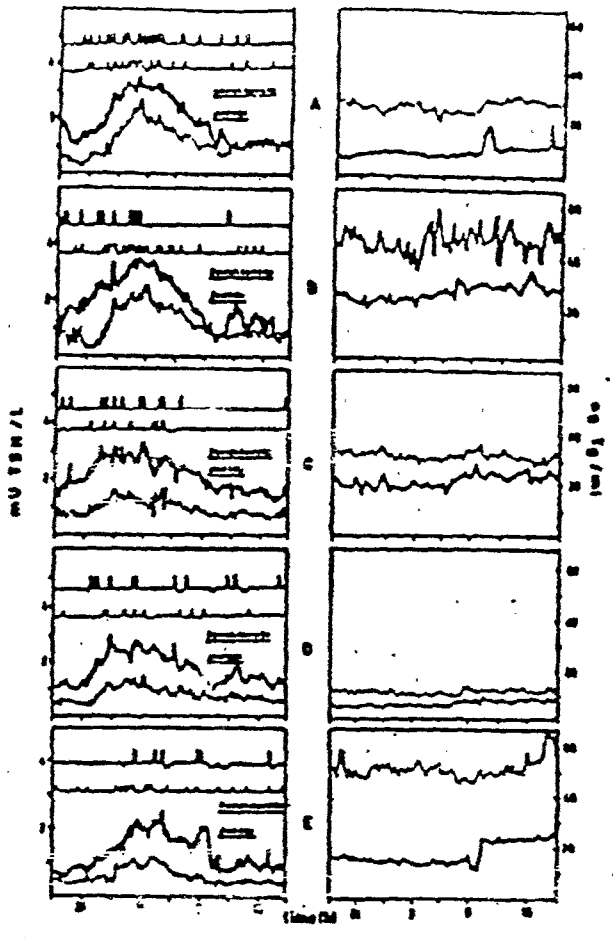

Fig 1. Individual pattern of TSH and Tg secretion in five healthy volunteers (A-E) following 4 weeks of treatment with 200 μg iodide/d PO (——) and perchlorate 3 × 300 mg/d PO (----). Blood sampling occurred every 10 minutes for TSH and every 30 minutes for Tg. The location of TSH pulses with the DESADE program is graphically shown in the upper part of each individual graph.

alimentary iodine deficiency are not well investigated. In-vitro and in-vivo test situations in animal models and in man generally focus either on the TSH-dependent thyroid function following iodine exposure that is 100-fold to 1,000-fold higher than expected, when changing from nor-

Table 1. Comparison of Thyroid Characteristics at the End of 4 Weeks' Supplementation With 200 μg Iodide/d PO and 4 Weeks' Iodine Depletion With 3 × 300 mg Perchlorate/d in Five Healthy Male Volunteers

	Iodide	p	Perchlorate
Thyroid volume (mL)	19.4 ± 7.2	NS	21.4 ± 8.8
Thyroidal iodide (mmol/mL)	4.0 ± 1.3	<.05	3.0 ± 1.2
T_3 (nmol/L)	2.4 ± 0.2	NS	2.4 ± 0.4
T_4 (nmol/L)	91.4 ± 10.2	NS	96.3 ± 10.3
FT_3 (pmol/L)	7.2 ± 1.5	NS	6.7 ± 0.8
FT_4 (pmol/L)	15.7 ± 1.2	.005	14.3 ± 2.0
rT_3 (nmol/L)	15.8 ± 4.7	NS	15.5 ± 4.8
Tg (ng/mL)	18.6 ± 10.8	.01	35.1 ± 14.9
TBG (μg/mL)	16.2 ± 1.3	NS	16.1 ± 3.8

NOTE. Results are means ± SD.

Table 2. Comparison of TSH Serum Levels and Characteristics of Pulsatile TSH Secretion at the End of 4 Weeks' Supplementation With 200 μg Iodide/d PO and 4 Weeks' Iodine Depletion With 3 × 300 mg Perchlorate/d in Five Healthy Male Volunteers

	Iodide	p	Perchlorate
Mean TSH serum levels (mU/L)	1.8 ± 0.3	.001	1.0 ± 0.1
Number of TSH pulses/24 h	11.4 ± 4.8	NS	14.6 ± 6.5
Amount of TSH secreted/pulse (mU/L)	0.5 ± 0.1	.008	0.3 ± 0.1
Temporal distribution of pulses			
8:00 PM to 4:00 AM	6.3 ± 4.0	NS	8.6 ± 2.8
4:00 AM to 12:00 PM	3.3 ± 1.3	NS	3.9 ± 1.8
12:00 PM to 8:00 PM	1.7 ± 0.9	NS	2.6 ± 1.5

NOTE. Results are means ± SD.

mal to iodine-deficient locations, or during a blockage of iodine organification severe enough to lead to hypothyroidism.[4,8,18,22] In our study, a high-normal dietary iodine supply to the thyroid was achieved by supplementation of iodine to the mildly deficient intake in the region, leading to a daily iodine intake approximately equivalent to that in the United States. This was compared with a mild experimental iodine deficiency elicited by perchlorate treatment and led to a pronounced decrease in TSH serum levels. These changes are in parallel to findings in rats undergoing decreased iodine intake that initially show no increase in levels of TSH,[20] whereas a more intense iodine deficiency leads to a continuous increase in TSH serum levels.[7,20] Comparative epidemiological studies in humans living in regions with mild-to-moderate dietary iodine deficiency and regions with normal iodine supply, either in Italy or northern Europe, showed a comparable reduction in TSH serum levels in iodine deficiency similar to our findings.[8,23] Development of thyroid autonomy has been suggested as the mechanism responsible for this reduction in serum TSH levels[21,23]; this interpretation is not supported by our data. Development of autonomy within 4 weeks in healthy subjects seems to be unlikely. The unchanged serum levels of total thyroid hormones and the slight decrease in FT_4 levels may be a very early sign of insufficient thyroid hormone synthesis. Our findings instead point toward a higher sensitivity of the thyroid to the effects of TSH under conditions of iodine depletion. By this mechanism, TSH would more effectively release thyroid hormones, and this would lead to a reduction of circulating serum TSH levels via negative feedback. Our recent investigation of 24-hour TSH rhythms in a group of nine patients with diffuse goiter grade I, and in one case grade II, supports this finding, as TSH serum levels in these patient groups were similarly reduced compared with healthy controls.[24]

The exact mechanism of iodine interference with thyroid function is unknown. It has been shown that a high iodine supply inhibits TSH effects on cAMP formation.[18,22] Changes from a low to high-normal iodine supply in the rat, in the absence of measurable changes in thyroid hormone or TSH plasma concentrations, result in structural alterations of the thyrocyte, modification of the basolateral transfer of iodine, formation of iodolactones,[25,26] and decreases in Tg iodination and endocytotic fluxes.[27-29] Opposite effects occurring as a first adaptive mechanism of the thyroid to iodine depletion are conceivable. Furthermore, Studer et al[29] provided evidence for a heterogeneous sensitivity of thyrocytes to TSH in euthyroidism. Recruitment of previously insensitive thyrocytes to TSH action may be another mechanism of adaptation in iodine deficiency, and may lead to an increased surface area for iodine trapping. Contributing to this recruitment may be an increase in thyroid blood flow, as suggested by recent detailed studies in rats and in humans showing an inverse relationship between thyroid blood flow and iodine.[30,31]

These observations are consistent with our results showing increased serum Tg levels. A participation of a higher percentage of thyrocytes in thyroid hormone synthesis and secretion in iodine deficiency may lead to an increased release of Tg stored in thyroid follicles. Decreasing follicular stores of Tg and therefore of Tg-bound iodine in iodine deficiency, together with an increased efficacy in trapping iodine, may be an early physiological mechanism of adaptation to iodine deficiency.[22,28] Alternatively, an increased de-novo synthesis of Tg may occur, and the iodine content of this newly formed Tg may be decreased in iodine deficiency. Since there are currently no assays available to determine the iodine content of Tg in circulation, this hypothesis cannot be further evaluated. Follicular necrosis, as discussed previously,[32] may serve as another explanation in our volunteers, but the consistency of thyroid hormone levels in these healthy subjects argues against such an explanation. Finally, we could not confirm earlier findings of a direct correlation between serum Tg levels and thyroid weight, serum thyroid hormone concentrations, or the T_3/T_4 ratio.[33]

Computer-assisted analysis of the pattern of TSH secretion demonstrated that the decrease in mean 24 hour TSH serum levels was due to a reduction in pulse amplitude, but no change in the frequency of TSH pulses or their distribution was found. Since the thyroid volume of the volunteers was unaltered by treatment, these data are not necessarily in contradiction to our initial hypothesis of an altered specific temporal pattern of TSH stimulation of thyroid proliferation. The decrease in TSH serum levels may occur as a very early sign of adaptation of the thyroid to iodine deficiency, because the sensitivity of thyrocytes is increased and a greater number of thyrocytes participate in thyroid function, thus reducing the requirement of pituitary TSH stimulation. A further decrease in dietary iodine supply or a longer-lasting iodine deficiency may then alter serum concentrations of TSH and/or the pulsatile pattern of TSH secretion potentially via a decrease in thyroid hormone synthesis, subsequently stimulating thyroid growth.

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